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Vinaya Sheela S
Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Science, PVNR TVU, Hyderabad, Telangana, India

G Purushotham
Associate Dean, College of Veterinary Science, PVNR TVU, Hyderabad, Telangana, India

D Pramod Kumar
Professor and University Head, College of Veterinary Science, PVNR TVU, Hyderabad, Telangana, India

M Lakshman
Retired Professor, Head and IOC Ruska Labs, Department of Veterinary Pathology, College of Veterinary Science, PVNR TVU, Hyderabad, Telangana, India

Corresponding Author:
Vinaya Sheela S
Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Science, PVNR TVU, Hyderabad, Telangana, India

Anatomical features of hair shaft of Asian elephant (*Elephas maximus*)

Vinaya Sheela S, G Purushotham, D Pramod Kumar and M Lakshman

Abstract
The shaft of guard hair in Elephant comprised of cuticle and cortex in all the samples examined. Medulla was present in the hair shaft of back region and tail region. The cuticle pattern was smooth and colored while cortex pattern at tip was smooth whereas it was coarse in middle and base regions in all hair samples observed.
Cortex color varied from light brown to dark brown color. Cross sectional shape was round in hair of all the regions examined. Mean shaft diameter of neck was 244.80±6.10 µm and that of tail was 253.95±3.56 µm which varied significantly. Imbricate scale pattern with closely placed scales and were not overlapped. Imbricate scale pattern with rippled scale margins were observed in Scanning Electron Microscopic (SEM) studies. The number of scales per 100 µm length of the shaft were in range of 12 to 17. Scale width ranged between 5.87 µm to 10.90 µm.

Keywords: Hair, elephant, medulla, imbricate, scale pattern

Introduction
Based on various features like cuticular pattern, cortex and medulla of hair, different species of animals can be identified. It is strongly resistant to decomposition (Lungu *et al*., 2007) [13] and is stable under adverse conditions (Kshirsagar *et al*., 2009) [12]. The morphological characteristics of hair vary from species to species which is an important tool for identification of animal species (Davis, 2010) [4]. Therefore, the applied aspects of hair are significantly important in biomedical studies. Elephant is an animal of regional importance of South India and the population of Asian elephants has been reduced in size and became highly fragmented during the past 3000-4000 years (Fleischer *et al*., 2001) [7]. Elephant hair is commonly used in the manufacture of jewelry and artefacts that are often sold illegally in the international wildlife trade (Yates *et al*., 2010) [17].

Materials and Methods
Guard hair samples were collected from head, neck, back, forelimb, hindlimb, lateral abdomen and tail regions were cut into three parts *viz*., tip, mid and basal parts and cleaned in soap solution followed by rinsing in distilled water for two to three times for removal of dirt and debris if any. Later they were cleaned in a mixture of equal volume of Ether and Alcohol for 2 to 3 minutes and blot dried. Samples were treated with hydrogen peroxide for two hours (Kshirsagar *et al*., 2009) [12] and cleared with Xylene and were dried on filter paper. Each hair was then mounted on microscopic slide in a drop of DPX (Xylene and DinButyl Phthalate) and covered with cover slip and allowed to dry for 48 hours for light microscopic observation. DPX was used instead of synthetic resin for mounting which was a slight modification to the method given by Drury and Wallington (1967) [6].
Cross sections were obtained by mounting the clean hair samples on tissue holder in standard embedding fluid and sections of 20 µm thick sections were cut using cryostat at -20° C.

Scanning Electron Microscope (SEM)
For SEM studies, washed hair samples were mounted over stubs with double sided carbon conductivity tape. A thin layer of gold coating was done over the samples by using an automated sputter coater (Model–JEOL-1600) for three minutes and immediately scanned under Scanning Electron Microscope (SEM–Model: JOEL-JSM 5600) at required magnification as per the standard protocol (Bozzola and Russell.,1998) [2].

Micrometry

Micrometrical studies were carried out by factorizing occular and Stage micrometer (Kshirsagar *et al*., 2009) [12].

Results and Discussion

Microscopic structure

Hair of Asian elephant revealed two distinct parts, a hair bulb and a hair shaft. The hair shaft consisted of cuticle and cortex (Fig.1) which differed from typical hair which comprised of cuticle, cortex and medulla (Houck *et al*., 2004) [8]. The cortex formed the bulk of the hair shaft as reported by Rowe, (2010) [15]. The color of hair in all the regions of the body were dark brown in the mid shaft but it was faint towards tip and base. The tip ends were rounded (Fig.2) in all the regions but broken tips were observed in hair of back and forelimb regions (Fig.3). These findings were in total agreement with the observations of Bisbing (1985) [1] who stated that the tip of hair varied greatly in morphology. The cuticle was thin translucent layer with smooth pattern on the exterior surface of the shaft which is similar to the reports made by Jaqueline and Genoways (1978) [9].

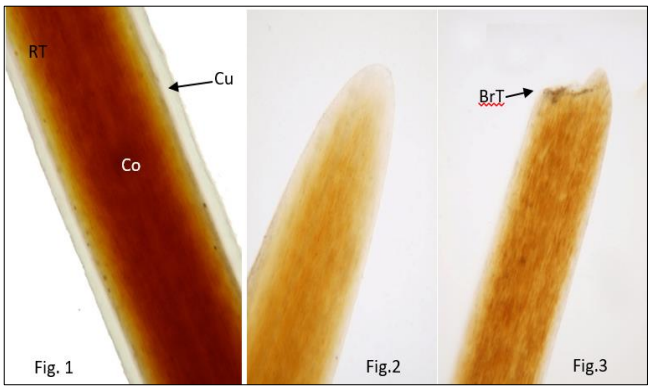

Fig 1: Midshaft showing cuticle (cu) and cortex (co).
Fig 2: The hair showing rounded tip
Fig 3: Tip showing broken edge (BrT)

The cortex pattern was coarse with Pigment granules**.** Pigment distribution was uniform with streaky appearance in few samples. The intensity of pigmentation was less towards tip and base (Fig.5) which was justified by Kennedy (1982) [10] who interpreted that tip of hair had proximal color demarcation. Brown colored pigmented granules were distributed all along the length of the shaft in hair of all the six regions which was supported by Zafarina and Sundararajulu (2009) [18] who stated that Eumelanin and Pheomelanin was found which imparts brown or black and yellow or red color respectively. Tip of lateral abdomen and hindlimb hair consisted of cortical fusi and ovoid bodies (Fig. which were small air spaces and irregular dark cavities respectively (Deedrick and Koch, 2004) [5].

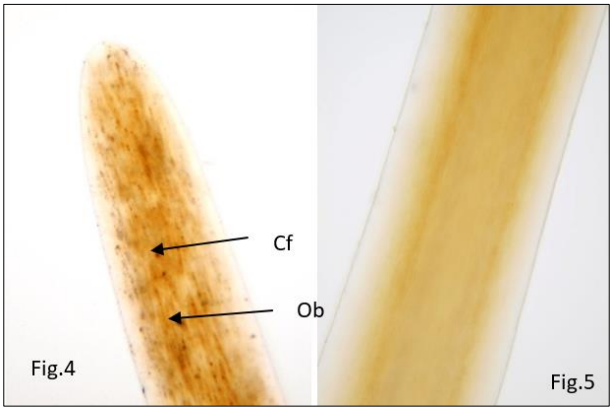

Fig 4: Tip of hair shaft showing Cortical fusi (Cf) and (Ob)
Fig 5: Base of haishowing showing low intensity of pigment with faint color

Medulla was not evident in hair of all regions except tail region which showed continuous medulla (Fig. 6) in midshaft of which was similar to medulla in lion hair (Wiley, 2004) and discontinuous medulla towards the tip (Fig. 7). Multiple medullae were observed in clutch hair of the tail (Fig.8).

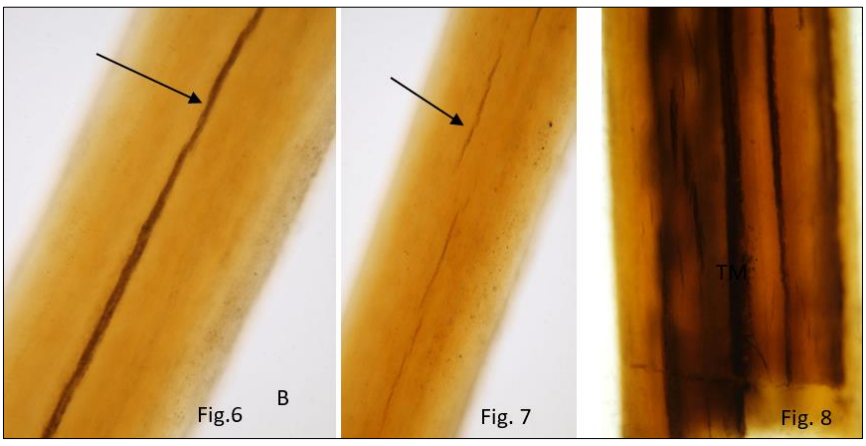

Fig 6: Midshaft of tail region showing continuous medulla (Arrow)
Fig 7: Tip of tail hair with faint discontinuous medulla (Arrow)
Fig 8: Tail clutch hair showing multiple medulla

Cross sections of hair shaft

Cross sectional shape was round (Fig.9) in hair of all regions which were similar to the studies of Sarkar *et al*., (2011) [16] in primates where he observed round to oval shaped hair shaft. Medulla was not evident in hair of all regions but was present in hair of back region (Fig.10). The cortex in cross sections revealed low pyramidal keratinized cortical cells. Cross section of hair shaft of back region revealed multiple medulla (Fig.11).

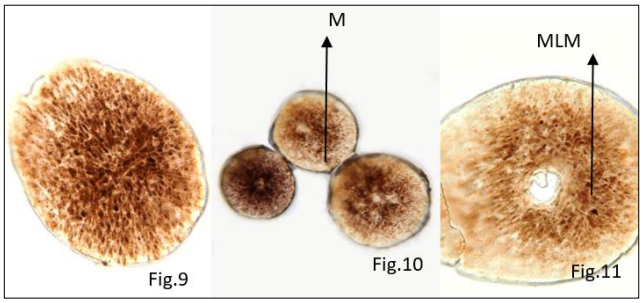

Fig 9: Cross section of hair shaft with low pyramidal keratinised cells
Fig 10: Cross section of back region midshaft of hair showing medulla
Fig 11: Cross section of hair showing multiple Medulla (MLM)

Micrometry

The shaft diameter of tip was less (121±6.16 µm) and was more in base (332.88±6.16). Difference was highly significant between the hair of neck (182.48±3.54µm) and tail regions (253.95±3.56 µm) whereas it was not significant between the hair of neck and forelimb and between back and tail regions. These findings are in acceptance with Chakraborty and De (2005) [3] who opined that the diameter of mammalian hair was not specific and varied from root to tip. Mean shaft diameter towards tip, mid shaft and base of hairs differed significantly (Table.1).

Table 1: Mean diameter of shaft in hair of elephants

	Shaft diameter	
Effect	**Mean (µm)**	**SE**
Overall	220.37	1.43
Neck	182.48[a]	3.54
Back	252.99[d]	3.56
Lateral abdomen	209.98[b]	3.56
Forelimb	183.13[a]	3.25
Hindlimb	239.67[c]	3.56
Tail	253.95[d]	3.56
Location		
Tip	172.95[a]	2.52
Middle	218.99[b]	2.40
Base	269.16[c]	2.51
Region × Location		
Neck - Tip	121.55	6.16
Midshaft	244.80	6.10
Base	181.10	6.16
Back - Tip	182.65	6.22
Midshaft	243.43	6.10
Base	332.88	6.16
L. abdomen-Tip	168.04	6.16
Midshaft	177.50	6.16
Base	284.40	6.16
Forelimb-Tip	113.33	6.16
Midshaft	178.25	4.35
Base - Tip	257.80	6.16
Hind limb- Tip	198.20	6.16
Midshaft	217.80	6.16
Base	303.00	6.16
Tail-Tip	253.90	6.16
Midshaft	252.16	6.16
Base	255.80	6.16

Means with similar superscript under each effect do not differ significantly

Scanning Electron microscopic studies

Scanning electron microscopy showed no air vesicles which indicated absence of medulla (Fig.13). The scale pattern was imbricate (Fig. 14) where the scales were with closely placed and were not overlapped. The margins of the scales were rippled throughout the length of the shaft as per the classification of Marinis and Asprea (2006) [14] who classified the structure of scale margins as smooth, slightly rippled, rippled or heavily rippled. The number of scales per 100 µm length of the shaft ranged between11.60 to 17.36. Scale width ranged between 5.87 µm to 10.90 µm (Fig.15).

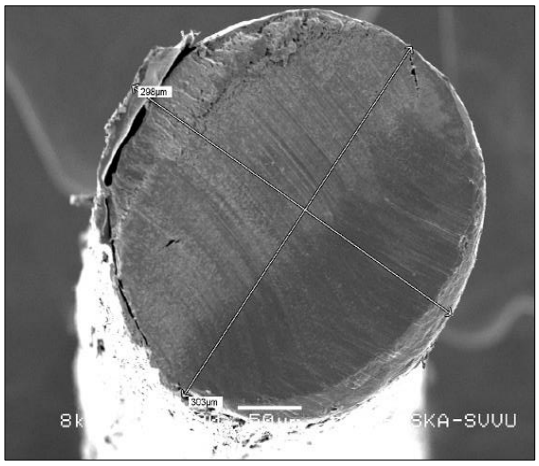

Fig 14: Scanning electron microscopy of cross section of hair shaft showing no air vesicles and medulla

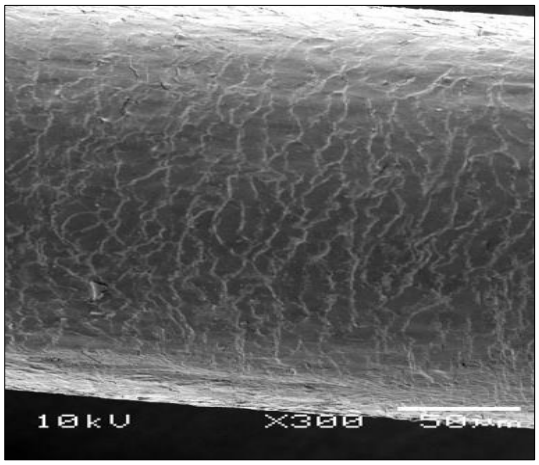

Fig 14: Scanning electron microscopy of elephant hairshaft showing imbricate scale pattern

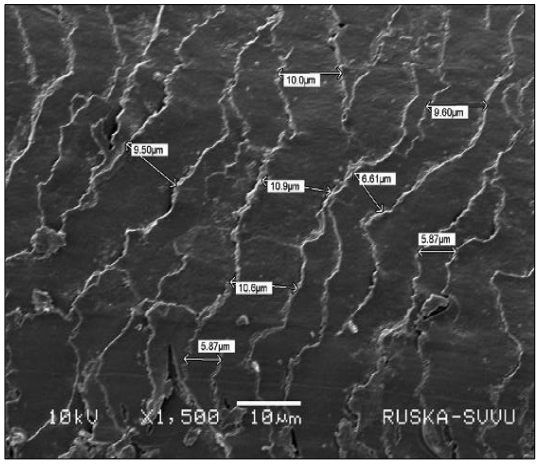

Fig 15: SEM showing rippled scale margins

Conclusion

The hair shaft of Asian elephant was mainly comprised of cuticle and cortex. Medulla was seen only in hair of back, tail and tail clutch. Multiple medulla was the characteristic feature of tail clutch hair. The cross sections were round with radiating cortical cells. The elephant hair was more in diameter than the prescribed range of an animal hair. These features of hair shaft are key tool in identification of elephants as its hair is used in jewelry and also they are being hunted for tusks.

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